Erythropoietin protects from posttraumatic edema in the rat brain: MRI and gravimetric studies

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Introduction: Numerous compounds have shown neuroprotective effects in experimental models of traumatic brain injury (TBI), but there is no pharmacological agent specifically aimed at blocking the progression of diffuse brain edema in the clinical setting. Therapy applied to patients with diffuse brain edema is still purely symptomatic [1]. In this study, because erythropoietin (Epo) is gaining interest in various neurological insults as a possible neuroprotective agent [2-7], we wondered whether posttraumatic administration of Epo could affect the course of brain edema in a rodent model of diffuse TBI.

Methods: In both MRI and a gravimetric experiments, 3 groups of rats (male wistar rats, 350-400 g) were studied: a first group was subjected to TBI and a saline sham treatment (TBI-saline, n = 12). A second group was treated with Epo 30 min after TBI (TBI-Epo, n = 12). A third group was sham-operated, receiving either saline (n = 6 rats) or Epo (n = 6), without TBI. Head trauma was induced according to the impact-acceleration model [8].Physiological parameters were controlled with the following criteria for exclusion: MABP <80 mmHg, PaO2 <100 mmHg, PaCO2 >45 mmHg, tHb <80 g/L. The reference time (H_0) corresponded to the TBI impact (TBI-Epo and TBI-saline) or equivalent time (sham-operated). Each group was i.v. administered at 30 min after H_0, either 0.5 ml of an isotonic saline solution, or 5,000 IU/kg of Epo diluted in 0.5 ml of saline solution. The sham-operated rats were treated similarly.

Diffusion weighted images (T_2-weighted spin-echo, TR = 2,000 ms; TE = 80 ms, matrix of 128×66) as well as T_1 maps (Inversion recovery FLASH, TR = 10 s; TE = 3.2 ms, matrix of 64×64) were acquired with a field of view of 30×30 mm^2 hourly during 6 hours, from H_1 to H_6. Diffusion sensitization was obtained with a b-factor of 500 s/mm^2 (δ = 9 ms; Δ = 20 ms). Measurements were averaged from experiments performed with diffusion gradient applied in each of the 3 main spatial directions (x, y, z). Acquisition of the set of diffusion weighted images lasted about 15 min. For T_1 maps, 12 inversion times ranging between 100 and 6,000 ms were used. The inversion pulse was specially non-spatially-selective in order to minimize in-flow contributions to the signal. Acquisition of each T_1 map lasted about 5 min.

Gravimetric measurements, were determined 6 hours after TBI. After the sacrifice of the rat, the brain was removed and samples (20-40 mg each) were taken from each hemisphere from the neocortex (n = 4) and from the caudoputamen (n = 3). The brain water content (BWC) was then calculated according to the equation: BWC (%) = (462.6 / SG) - 362.6, where SG is tissue specific gravity.

All data are expressed as mean ± SD. Statistical analysis during the time course was performed with two-way analysis of variance for repeated measurements. Each value was compared to the reference time (H_0: physiological data, H_1: MRI measurements) using the Scheffé F test. Comparisons between the groups of rats were subjected to factorial analysis of variance using the Scheffé F. Statistical significance was declared when $p < 0.05$.

Results: During the experiment, the two TBI groups had comparable physiological data. No MRI evidence for intracerebral hemorrhage was found at the site of impact. No significant apparent diffusion coefficient (ADC) or T_1 changes over time were found within each group of rats. ADC values ($\times 10^{-3}$ mm^2/s) in the neocortex as well as in the caudoputamen were significantly lower in the TBI-saline group: (0.61 ± 0.13 vs. 0.73 ± 0.11 for TBI-Epo and 0.71 ± 0.09 for sham-operated) and (0.60 ± 0.19 vs. 0.74 ± 0.10 for TBI-Epo and 0.79 ± 0.14 for sham-operated) respectively. Intergroup analysis indicated significant differences between the TBI-saline group and the two other groups at the different measurement times of the experiment (Fig 1). No differences in ADC were found between the TBI-Epo and the sham-operated groups. T_1 values (ms) were significantly longer in the TBI-saline group than in the TBI-Epo group in neocortex and in caudoputamen: 1821 ± 62 vs. 1782 ± 85 and 1745 ± 119 vs. 1676 ± 81, respectively ($p<0.01$) (Fig 2). No differences in T_1 values were found between the TBI-Epo group and the sham-operated group. BWC measurements in neocortex and in caudoputamen were significantly lower in the TBI-Epo group than in the TBI-saline group: 78.2 ± 0.3% vs. 79.1 ± 0.4% and 78.0 ± 0.3% vs. 78.6 ± 0.5%, respectively ($p < 0.01$) (Fig 3). No differences in BWC were found between the TBI-Epo group and the sham-operated group.

Conclusion: ADC, T_1 and BWC measures indicate that posttraumatic administration of Epo can significantly reduce the development of brain edema in a model of diffuse TBI. These findings add to the accumulating evidence of beneficial effects of Epo and offer new perspectives in the treatment of TBI. Further studies should be conducted to identify the biochemical mechanisms involved in these immediate effects.

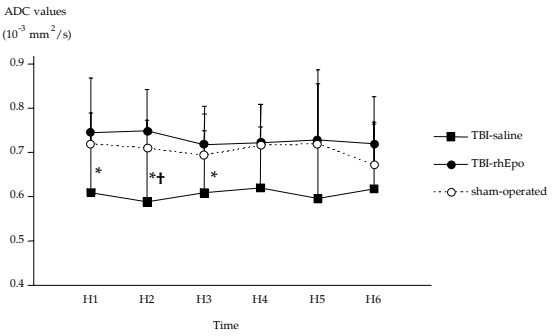

Figure 1: Time course of ADC values in neocortex in the 3 groups of rats. (caudoputamen: same results, not shown)

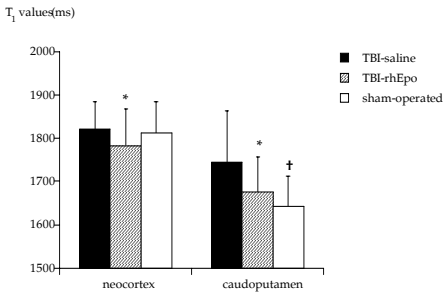

Figure 2: Quantitative T_1 mean values in neocortex and in caudoputamen during the entire experiment in the 3 groups of rats.

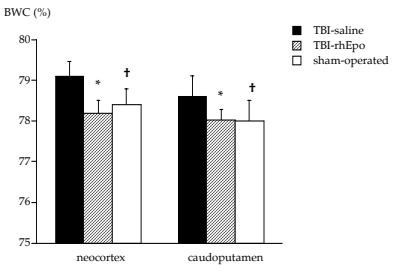

Figure 3: Brain water content (BWC) in neocortex and in caudoputamen six hours following TBI in the 3 groups of rats.

References: [1] Narayan RK et al, J Neurotrauma 2002. [2].Bernaudin M et al, JCBFM 1999. [3].Brines ML et al, PNAS USA 2000.[4].Siren AL et al, PNAS USA 2001. [5].Springborg JB et al, Br J Pharmacol 2002. [6].Gorio A et al, PNAS USA 2002. [7].Grasso G et al, PNAS USA 2002. [8].Marmarou A et al, J Neurosurg 1994.